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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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☐ *Additional inventors are being named on the 1 separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

DEFINED DOSE THERAPEUTIC PHAGE

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 51 ☐ CDs, Number

☐ Drawing(s) *Number of Sheets* ☐ Other (specify)

☒ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

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FILING FEE AMOUNT ($)

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: .

Respectfully submitted,

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REGISTRATION NO. 51,868 *(if appropriate)*

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PROVISIONAL

PATENT APPLICATION

DEFINED DOSE THERAPEUTIC PHAGE

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DEFINED DOSE THERAPEUTIC PHAGE

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] NOT APPLICABLE

FIELD OF THE INVENTION

[0002] The invention provides therapeutic, defined-dose anti-bacterial phage preparations, methods to make such preparations, methods to treat bacterial infections using such preparations and method to diagnose bacterial infections using such preparations.

BACKGROUND OF THE INVENTION

[0003] Bacteria are ubiquitous, and are found in virtually all habitable environments. They are common and diverse ecologically, and find unusual and common niches for survival. They are present all around the environment, and are present in soil, dust, water, and on virtually all surfaces. Many are normal and beneficial strains, which provide a synergistic relationship with hosts. Others are not so beneficial, or provide problems along with benefits.

[0004] Pathogenic bacteria can cause infectious diseases in humans, in other animals, and also in plants. Some bacteria can only make one particular host ill; others cause trouble in a number of hosts, depending on the host specificity of the bacteria. The diseases caused by bacteria are almost as diverse as the bacteria themselves and include food poisoning, tooth ache, anthrax, and even certain forms of cancer. These are typically the subject of the field of clinical microbiology.

[0005] Bacteria are killed in nature by bacteria-specific viruses, *e.g.*, bacteriophage. Pyosins are believed to by tail-like portions of tailed phages. *See e.g.*, Nakayama, *et al.* (2000) *Mol. Microbiol.* 38:213-31; Traub, *et al.* (1996) *Zentralbl. Bakteriol.* 284:124-35; Ito, *et al.* (1986) *J. Virol.* 59:103-111; Rocourt (1986) *Zentralbl. Bakteriol. Mikrobiol Hyg.* 261:12-28; Shinomiya (1984) *J. Virol.* 49:310-14; and Ishii S, *et al.*, *J Mol Biol.*, 13, 428-431, (1965); Daw and Falkiner, *Micron*, 27(6), 467-479, (1996); Strauch *et al.*, *Appl. Environ. Microbiol.*, 67(12), 5635-5642, (2001); Abdelhamid *et al.*, *Appl. Environ. Microbiol.*, 68(11), 5704-5710, (2002).

[0006] Certain bacteria are normally innocuous, but become pathogenic upon presentation of the appropriate opportunity, or become problematic upon introduction to an abnormal site or situation. Persons lacking effective immune systems are most vulnerable, and certain bacteria use susceptible weak hosts to provide a temporary environment to proliferate and disperse throughout the population.

[0007] Statistically, infectious diseases are a major medical problem. *See, e.g.*, Watstein and Jovanovic *Statistical Handbook on Infectious Diseases* Greenwood, ISBN: 1573563757 (2003). In the U.S., some 40-70K deaths result from bloodstream nosocomial (hospital derived) infections each year.

[0008] Synthetic chemical antibiotics have been used to treat bacterial infections for many years, and have minimized the frequency and effects of many infectious diseases. Antibiotics had about $32B worldwide sales in 2002. A great need exists for continued antimicrobials. The present invention solves these and other problems.

BRIEF SUMMARY OF THE INVENTION

[0009] The present invention provides methods of using a host bacterium to produce an anti-bacterial phage that inhibits growth of a target bacterium and is unable to replicate in the target bacterium.

[0010] Replication of the anti-bacterial phage can be prevented by inactivating a nucleic acid of the anti-bacterial phage. Nucleic acid inactivation can be performed by nicking the nucleic acid, fragmenting the nucleic acid, cross-linking said nucleic acid, or by chemically modifying said nucleic acid.

[0011] Replication of the anti-bacterial phage can be prevented by removing a nucleic acid from the anti-bacterial phage. Nucleic acid can be removed by osmotic shock, by a freeze thaw cycle, by chemical methods, or by mechanical methods.

[0012] Replication of the anti-bacterial phage can be prevented where the anti-bacterial phage comprises a mutation and cannot assemble into a replication competent phage in the target bacterium. In this embodiment the host bacterium is a complementing host bacterium that is able to complement the mutation of said anti-bacterial phage and allow replication and production of said anti-bacterial phage in the complementing host bacterium. In one embodiment, the mutation is a temperature sensitive mutation and the host bacterium

complements the mutation at the non-permissive temperature. In another embodiment a second phage is used to complement the mutation.

[0013] The present invention also provides complementing host bacterium and complementing phage for use in production of anti-bacterial phage.

[0014] The present invention provides pharmaceutical compositions comprising an anti-bacterial phage that inhibits growth of a target bacterium, and has diminished replication activity in the target bacterium. In one embodiment the antibacterial phage has no replication activity in the target bacterium. In another embodiment the antibacterial phage kills the host bacterium.

[0015] In a further embodiment of the pharmaceutical composition, the anti-bacterial phage has less than 20% of the a nucleic acid content of an intact parental phage. In another embodiment, the anti-bacterial phage does not contain detectable nucleic acid.

[0016] In one embodiment, anti-bacterial phage is an intact phage having nucleic acid with reduced a replication capacity. In another embodiment, the anti-bacterial phage comprises a tail portion of a tailed phage. In a further embodiment, the anti-bacterial phage consists essentially of a tail portion of a tailed phage.

[0017] In one embodiment, the pharmaceutical composition also includes a second anti-microbial agent. The second microbial agent can be, *e.g.*, an antibiotic or a second anti-bacterial phage. In another embodiment, the pharmaceutical composition also includes a therapeutically compatible buffer or excipient.

[0018] In one aspect the present invention provides methods of making an anti-bacterial phage. Anti-bacterial phage can be made *e.g.*, by amplifying a phage in a host bacterium, harvesting the phage from the bacterial culture, and removing substantially all of the nucleic acids from the phage, thereby producing the anti-bacterial phage. Anti-bacterial phage can be made *e.g.*, by amplifying a phage in a host bacterium, harvesting the phage from the bacterial culture, and inactivating the nucleic acids of the phage, thereby producing the anti-bacterial phage. Anti-bacterial phage can be made *e.g.*, by amplifying a phage in a host bacterium, harvesting the phage from the bacterial culture, harvesting the phage from the bacterial culture before an intact phage is produced, thereby producing the anti-bacterial phage. Anti-bacterial phage can be made *e.g.*, by amplifying a phage in a host bacterium, harvesting the phage from the bacterial culture, and harvesting the phage from the bacterial culture, wherein

a nucleic acid of said anti-bacterial phage comprises a mutation and cannot assemble into a replication competent phage, and wherein the host bacterium is a complementing host bacterium that is able to complement the mutation of said anti-bacterial phage and allow replication of said anti-bacterial phage in the complementing host bacterium, thereby producing the anti-bacterial phage.

[0019] The present invention provides a method of treating a bacterial infection in a subject by administering a therapeutically effective amount of a pharmaceutical compound including an anti-bacterial phage. In one embodiment, the bacterial infection is caused by the target bacterium, *e.g.*, *E. coli.* The subject of treatment can be a human, a primate, a food animal, or a companion animal. A second antimicrobial agent, *e.g.*, an antibiotic or a second anti-bacterial phage, can be administered with the anti-bacterial phage composition. The pharmaceutical composition can be administered systemically, *e.g.*, parenterally or orally, or can be administered topically or by inhalation.

[0020] The present invention provides a pharmaceutical composition including a genetically incompetent anti-bacterial phage, that inhibits growth of a target bacterium. In one embodiment, the genetically incompetent anti-bacterial phage lacks a full complement of genetic material. In another embodiment, the genetically incompetent anti-bacterial phage has a mutation and cannot assemble into a replication competent phage in the target bacterium. In a further embodiment, the genetically incompetent anti-bacterial phage comprises nucleic acid with a reduced replication capacity. In an additional embodiment, the genetically incompetent anti-bacterial phage consists essentially of a tail protein from a tailed phage.

[0021] The present invention also provides a method of using a pharmaceutical composition including a genetically incompetent anti-bacterial phage to treat a bacterial infection by administering a therapeutically effective amount of the pharmaceutical composition including the genetically incompetent anti-bacterial phage. The method can be used to treat a human, a primate, a food animal, or a companion animal. The pharmaceutical can be administered systemically, *e.g.*, parenterally or orally, or can be administered topically or by inhalation. A second antimicrobial agent, *e.g.*, an antibiotic or a second anti-bacterial phage, can be administered with the anti-bacterial phage composition.

[0022] In one aspect the present invention provides a method of identifying an anti-bacterial phage that is unable to replicate in a target bacterium by identifying a target

bacterium, identifying a phage that can inhibit growth of the target bacterium, and determining a form of the phage that is unable to replicate in the target bacterium. In another aspect the present invention provides antibacterial-phages that have been identifies using this method.

[0023] In another aspect the present invention provides a method of producing an anti-bacterial phage by amplifying a phage, *e.g.*, an intact parental phage, in a host bacterium, harvesting the phage from the bacterial culture, and removing substantially all of the nucleic acids from the phage, thereby producing the anti-bacterial phage. The nucleic acids can be removed by a variety of methods, *e.g.*, osmotic shock, freeze thaw cycle, chemical methods, or mechanical methods.

[0024] In one embodiment, the antibacterial phage is a tailed phage. Anti-bacterial phages can be produced from tailed phage by separating a tail from a head of the tailed phage, and isolating the tail, thereby removing substantially all of the nuclei acids from the tailed phage. Alternatively, anti-bacterial phages can be produced from tailed phage by harvesting the phage before a head and a tail have assembled to form an intact phage, and isolating the tail, thereby removing substantially all of the nuclei acids from the tailed phage.

[0025] In one embodiment the present invention provides a method of making a defined dose anti-bacterial phage that kills a target bacterium, by using at least one of the following steps: producing said anti-bacterial phage in a host bacterium and isolating tail portions from DNA containing heads; producing said anti-bacterial phage in a host bacterium and inactivating nucleic acid of said phage; producing the anti-bacterial phage in a host bacterium and harvesting before complete assembly; producing the anti-bacterial phage in a complementing host; producing the anti-bacterial phage in a host bacterium with a helper phage; or providing the anti-bacterial phage in a permissive host which phage are non-permissive in a different temperature. The method can be performed using a tailed phage, and, for example, isolating the antibacterial phage tails separation of phage tails from DNA containing phage heads. DNA can be inactivated by using at least one of the following methods: nicking said nucleic acid; fragmenting said nucleic acid; cross-linking said nucleic acid; chemically modifying said nucleic acid; removing said nucleic acid; or harvesting said phage before complete assembly has occurred.

[0026] Where the anti-bacterial phage is produced in a complementing host, the anti-bacterial phage can include any of the following mutations a point mutation; a deletion

mutation; or an insertion mutation in a gene necessary for replication in said target bacterium. The complementing host will provide the function of the mutated gene product and the present invention also provides the complementing host bacterium. The present invention also provides method to produce defined dose anti-bacterial phage that exhibit diminished capacity to transmit toxin genes in the target bacteria when compared to intact phage in the host bacterium, as well as defined dose anti-bacterial phage that have diminished immunogenicity compared to intact phage from a host bacteria upon administration to a mammal.

[0027] In another embodiment, the present invention provides defined dose anti-bacterial phage that exhibits no detectable replication activity in the target bacterium.

[0028] The defined dose anti-bacterial phage can be used to treat target bacteria, *e.g.*, a pathogenic bacterium, such as a nosocomial or pyogenic bacterium. In one embodiment, the pathogenic bacterium is a gram negative bacterium, *e.g.*, an *E. coli* bacterium.

[0029] The present invention also provides a defined dose anti-bacterial composition comprising a phage protein derived from an intact parental phage, where the anti-bacterial composition kills a target bacterium, and composition exhibits less than 20% replication activity or less than 5% replication activity in the target bacterium, when compared to the intact parental phage. In one embodiment, the anti-bacterial phage exhibits diminished capacity to transmit toxin genes in the target bacteria when compared to intact phage in the host bacterium. In another embodiment, the anti-bacterial phage exhibits diminished immunogenicity compared to intact phage from a host bacteria upon administration to a mammal. In a further embodiment, the anti-bacterial phage exhibits no detectable replication activity in the target bacterium.

[0030] The defined dose anti-bacterial composition can be to kill a target bacterium that is a pathogenic bacterium. Examples of pathogenic bacterium include nosocomial or pyogenic bacterium, gram negative bacterium, and *E. coli* bacterium.

[0031] The defined dose anti-bacterial composition comprising a phage protein can also include a nucleic acid with reduced replication capacity, *e.g.*, nicked, fragmented, cross linked, or UV irradiated. The defined dose anti-bacterial composition comprising a phage protein can also include less than 20% of the nucleic acid content of the intact parental phage; can lack any detectable nucleic acid; or can include damaged DNA that is unable to be replicated.

[0032] The defined dose anti-bacterial composition comprising a phage protein can be a tail derived from an intact parental phage that is a tailed phage.

[0033] The defined dose anti-bacterial composition comprising a phage protein can include a second anti-microbial agent, *e.g.*, an antibiotic, a bacterial cell wall growth disrupting compound, or a second anti-bacterial phage. The defined dose anti-bacterial composition comprising a phage protein can also include a therapeutically compatible buffer or excipient.

[0034] The defined dose anti-bacterial composition comprising a phage protein can be made by a processing the intact parental phage to remove or inactivate nucleic acids; or by harvesting phage from a host bacterium before intact phage are assembled; or can by made in a complementing host strain, where the parental strain is defective in expressing critical genes for assembly, production, release, or infection by said phage. The defect can be a result of a point mutation, an insertion, or a deletion that prevents phage replication.

[0035] The defined dose anti-bacterial composition comprising a phage protein can be administered to a eukaryote suffering from a bacterial infection by the target bacterium. The eukaryote can be a mammal, including a primate.

[0036] The target bacterium can by a pathogenic, nosocomial, or pyogenic bacterium. In one embodiment, the target bacterium is *E. coli*. In one embodiment, the infection has been diagnosed to be susceptible to the composition.

[0037] The defined dose anti-bacterial composition comprising a phage protein can be administered topically or with an antibiotic or other anti-microbial agent. In one embodiment, the infection has already been treated with an antibiotic. The eukaryote can also by inoculated with another bacterium to replace target bacterium.

[0038] The present invention provides a therapeutic anti-bacterial composition including a genetically incompetent phage that kills a target bacterium. The genetically incompetent phage can *e.g.*, lack detectable nucleic acid; lack a set of genes necessary to replicate in the target bacterium; include damaged nucleic acid that cannot be replicated; or include a tail protein from a tailed phage. The composition can be used therapeutically to treat a companion animal, food animal, or primate. The target bacterium can be a pathogenic bacterium. The composition can be administered topically or systemically. The composition can by administered in combination with a second anti-bacterial agent, *e.g.*, an antibiotic.

DETAILED DESCRIPTION OF THE INVENTION

I. INTRODUCTION

[0039] The present invention provides anti-bacterial phages that are unable to replicate in a target bacteria and that also inhibit growth of the target bacteria. The anti-bacterial phages are thus useful for inhibiting bacterial growth in the environment and for treating bacterial infection in a subject in need of such treatment. Because the anti-bacterial phages are unable to replicate in a target bacteria, they can be administered as a defined dose therapeutic composition for treatment of bacterial infections.

[0040] This invention provides the first disclosure that, for each pathogenic bacteria, a tailed portion of a phage from the *Siphoviridae* or *Myoviridae* families will be useful as a defined dose therapeutic agent to inhibit growth of or kill the pathogenic bacteria.

II. DEFINITIONS

[0041] As used herein an "anti-bacterial phage" is a phage or phage construct (*e.g.*, a phage tail, or tail fragment, a phage protein, or ghost phage) that is unable to replicate or assemble in a target bacterium, but that inhibits the growth of the target bacterium. Thus, an "anti-bacterial phage" can include a portion of a phage that can be used to inhibit growth of the target bacterium. For example, an antibacterial phage can be a portion of an intact phage that can be produced in a non-target bacteria. Thus, as defined herein, an anti-bacterial phage can include a structural portion of an intact phage, *e.g.*, a tail portion of a tailed phage; or an isolated protein component of an intact phage.

[0042] Those of skill will recognize that phage are viruses that infect bacteria. Anti-bacterial phages include a phage from the families *Podoviridae, Siphoviridae, Myoviridae,Lipothrixviridae, Plasmaviridae, Corticoviridae, Fuselloviridae, Tectiviridae, Cystoviridae, Levividae, Microviridae, Inoviridae plectrovirus*, and *Inoviridae inovirus*. In some embodiment the antibacterial phage is derived from a tailed phage from the families *Podoviridae, Siphoviridae*, and *Myoviridae*. In a typical embodiment the antibacterial phage is derived from a naturally occurring or wild-type tailed phage from the family *myoviridae* or from the family *Siphoviridae*.

[0043] As used herein, "target bacterium" or "target bacteria" refer to a bacterium or bacteria whose growth is inhibited by an antibacterial phage. "Growth inhibition" can refer to slowing of the rate of bacterial cell division, or cessation of bacterial cell division, or to death

of the bacteria. In a typical embodiment, the "target bacterium" or "target bacteria" are pathogenic bacteria.

[0044] As used herein, "host bacterium" or "host bacteria" refer to a bacterium or bacteria used to replicate or amplify a phage, sometimes referred to as a parental phage, that is used to produce an anti-bacterial phage. Host bacteria or bacterium are also referred to as "production host bacterium" or "production host bacteria," throughout. In one embodiment, the parental phage is a prophage.

[0045] An anti-bacterial phage is a phage that, in addition to its growth inhibitory activity, is unable to replicate in the target bacterium. As used herein, "replication" refers to production of a phage. As used herein, "replication" or "replication activity" refers to replication of genetic material, including nucleic acids, *e.g.*, DNA or RNA. Replication also includes the assembly of an intact phage, and includes synthesis of components of the phage, including proteins; and assembly of the components of the phage to form an intact phage. Components of the phage include *e.g.*, tails, heads, and nucleic acids. Replication typically leads to the production of "an intact phage," which is a phage that is able to replicate itself in a non-target bacteria. Thus, a replication deficient phage is a phage that is deficient in one or more of the processes noted above. Standard methods are conveniently used to evaluate the replication capacity of a construct. For example, the ability to form plaques on a host bacterial lawn can be used. Typically, the inactivation will decrease the replication capacity by at least 3 fold, and may affect it by 10, 30, 100, 300, etc., to many orders of magnitude.

[0046] Loss of replication activity by an anti-bacterial phage, (also referred to as being unable to replicate, loss of assembly activity, and genetically incompetent anti-bacterial phage), can occur, for example through removal of nucleic acids, inactivation of nucleic acids, removal of structural portions of a phage, *e.g.*, removal of the head of a tailed phage. The replication activity of an anti-bacterial phage in a target bacterium is preferably measured relative to the replication activity of the parental phage in the host bacterium, or relative the parental phage in the target bacterium. Thus, an anti-bacterial phage can exhibit less than 10%, 1%, 0.1%, 0.001% or 0.0001% of the levels of nucleic acid, *e.g.*, DNA or RNA, polymerase activity of a parental phage. Diminished polymerase activity can occur because of changes in the enzyme or changes in the substrate nucleic acids, *e.g.*, removal or inactivation of the nucleic acid of an anti-bacterial phage. In another embodiment the anti-bacterial phage, can have less than 10%, 1%, 0.1%, 0.001% or 0.0001% of the levels of a

component of the parental phage, *e.g.*, DNA or RNA, phage heads, or specific phage proteins.

[0047] Anti-bacterial phage can also include phage whose nucleic acids have been inactivated. Those of skill will recognize that many methods can be used to inactivate nucleic acids, *e.g.*, UV and X ray irradiation, fragmentation of DNA, treatment with chemicals including D-glucosamine and ferrous ammonium sulfate.

[0048] Anti-bacterial phage also include phage constructs whose nucleic acid has been partially or totally removed. Such phage are also referred to as "ghosts" or "ghost phage." Methods to remove nucleic acids from phage and make anti-bacterial phage include removal of all or substantially all of the structural components that contain phage nucleic acids, *e.g.*, retaining the tails of a tailed phage. Nucleic acid can also be removed by compromising the structural integrity of a phage, *e.g.* by osmotic shock with a salt or sugar; freezing and thawing the phage; and chemical treatments, including treatment with the following: LiCl or other salts, EDTA or other chelating agents, organic salts, amino acids, and reducing agents; and mechanical methods including the following: shearing, lyophilization, sonication, and microwave treatment.

[0049] Anti-bacterial phage also include phage that comprise a mutation and cannot assemble into a replication competent phage in the target bacteria. Mutations can include mutations in genes that encode enzymes for replication of nucleic acids or genes that encode regulators of replication; or in genes that encode structural components of a phage or genes that encode regulators of the synthesis of structural components. The mutations can be in the coding region of a gene or in a regulatory region of the gene, *e.g.*, a promoter. Such an anti-bacterial phage will be produced in a "complementing host bacterium." A complementing host bacterium comprises a complementing nucleic acid that complements the mutation comprised by the anti-bacterial phage. In some embodiments, the bacterium comprises a nucleic acid that encodes a protein that supplies the function of the mutated protein in the anti-bacterial phage. The complementing nucleic acid can be part of the bacterial genome or part of an extra-genomic element, *e.g.*, a plasmid. In one embodiment, a second phage in the bacterium comprises the complementing nucleic acid. Examples of phage mutations and complementing host or phage include phage comprising termination mutations and complementing host or phage comprising tRNA suppressors, phage comprising mutations in genes critical for replication and complementing host or phage comprising antisense

constructs that complement the mutation, phage comprising insertion mutations and complementing host or phage that comprise suppressors of the mutations, and phage comprising deletion mutations and complementing host or phage that comprise suppressors of the mutations.

[0050] An "anti-microbial agent" is an agent or compound that can be used to inhibit the growth of or to kill bacteria. Anti-microbial agents include antibiotics, chemotherapeutic agents, and anti-bacterial phages, usually referring to the second or more phages when more than one anti-bacterial phage is present in a compound or used in a method of the present invention.

[0051] As used herein, "amplifying a phage in a host bacterium" refers to infecting a host bacterium with a parental phage under conditions that allow the phage to replicate and make copies of itself. As used herein, "harvesting a phage from a bacterial culture" refers to removing the phage from the host bacterial culture. In some embodiments, the phage can have the attributes of an anti-bacterial phage, *e.g.*, ability to inhibit growth of the target bacterium and inability to replicate in the target bacterium. In other embodiments, the phage can be treated, before or after removal from the bacterial culture, to produce an anti-bacterial phage, *e.g.*, through removal or inactivation of nucleic acids. In a further embodiment, the antibacterial phage can be further purified before administration to a subject infected with the target bacteria.

[0052] A "bacterial infection" refers to growth of bacteria in a subject, such that the bacteria cause disease or symptoms in the subject.

[0053] A "subject in need of treatment" is a animal with a bacterial infection that is life-threatening or that impairs health or shortens the lifespan of the animal. The animal can be a fish, bird, or mammal. Exemplary mammals include humans and domesticated animals (*e.g.*, cows, horses, sheep, pigs, dogs and cats). In some embodiments antipabecterial phage are used to treat plants with bacterial infections.

[0054] A "pharmaceutically acceptable" component is one that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio.

[0055] A "safe and effective amount" refers to the quantity of a component that is sufficient to yield a desired therapeutic response without undue adverse side effects (such as toxicity,

irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention. By "therapeutically effective amount" is meant an amount of a component effective to yield the desired therapeutic response, *e.g.*, an amount effective to slow the rate of bacterial cell division, or to cause cessation of bacterial cell division, or to cause death of the bacteria. The specific safe and effective amount or therapeutically effective amount will vary with such factors as the particular condition being treated, the physical condition of the patient, the type of mammal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compounds or its derivatives.

[0056] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in single- or double-stranded form. The term encompasses nucleic acids containing nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs).

[0057] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0058] A particular nucleic acid sequence also implicitly encompasses "splice variants." Similarly, a particular protein encoded by a nucleic acid implicitly encompasses any protein encoded by a splice variant of that nucleic acid. "Splice variants," as the name suggests, are products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate

splicing of exons. Alternate polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Products of a splicing reaction, including recombinant forms of the splice products, are included in this definition.

[0059] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0060] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0061] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0062] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode a given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to the

corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0063] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0064] The following eight groups each contain amino acids that are typically conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)).

[0065] Macromolecular structures such as polypeptide structures can be described in terms of various levels of organization. For a general discussion of this organization, *see, e.g.*, Alberts *et al., Molecular Biology of the Cell* (3rd ed., 1994) and Cantor and Schimmel, *Biophysical Chemistry Part I: The Conformation of Biological Macromolecules* (1980). "Primary structure" refers to the amino acid sequence of a particular peptide. "Secondary structure" refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains, *e.g.*, transmembrane domains, pore domains, and cytoplasmic tail domains. Domains are portions of a polypeptide that form a compact unit of the polypeptide and are typically 15 to 350 amino acids long. Exemplary domains

include domains with enzymatic activity, *e.g.*, phosphatase domains, ligand binding domains, etc. Typical domains are made up of sections of lesser organization such as stretches of β-sheet and α-helices. "Tertiary structure" refers to the complete three dimensional structure of a polypeptide monomer. "Quaternary structure" refers to the three dimensional structure formed by the noncovalent association of independent tertiary units. Anisotropic terms are also known as energy terms.

[0066] A "label" or a "detectable moiety" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ^{32}P, fluorescent dyes, electron dense reagents, enzymes (*e.g.*, as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins which can be made detectable, *e.g.*, by incorporating a radiolabel into the peptide or used to detect antibodies specifically reactive with the peptide.

[0067] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0068] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0069] "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as

gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Typically, the antigen-binding region of an antibody will be most critical in specificity and affinity of binding.

[0070] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0071] Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'$_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'$_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'$_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990))

[0072] For preparation of antibodies, e.g., recombinant, monoclonal, or polyclonal antibodies, many technique known in the art can be used (*see, e.g.*, Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985); Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *Antibodies, A Laboratory Manual* (1988); and Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986)). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and

light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity (*see, e.g.*, Kuby, *Immunology* (3rd ed. 1997)). Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Patent 4,946,778, U.S. Patent No. 4,816,567) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized or human antibodies (*see, e.g.*, U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, Marks *et al.*, *Bio/Technology* 10:779-783 (1992); Lonberg *et al.*, *Nature* 368:856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14:845-51 (1996); Neuberger, *Nature Biotechnology* 14:826 (1996); and Lonberg & Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995)). Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990); Marks *et al.*, *Biotechnology* 10:779-783 (1992)). Antibodies can also be made bispecific, i.e., able to recognize two different antigens (*see, e.g.*, WO 93/08829, Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991); and Suresh *et al.*, *Methods in Enzymology* 121:210 (1986)). Antibodies can also be heteroconjugates, e.g., two covalently joined antibodies, or immunotoxins (*see, e.g.*, U.S. Patent No. 4,676,980 , WO 91/00360; WO 92/200373; and EP 03089).

[0073] A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

[0074] In one embodiment, the antibody is conjugated to an "effector" moiety. The effector moiety can be any number of molecules, including labeling moieties such as radioactive labels or fluorescent labels, or can be a therapeutic moiety. In one aspect the antibody modulates the activity of the protein.

[0075] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide or anti-bacterial

phage comprising a protein, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires an antibody that is selected for its specificity for a particular protein. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

III. PATHOGENIC BACTERIA

[0076] In a preferred embodiment, the anti-bacterial phage are used to inhibit growth of a pathogenic bacteria.

A. Natural, infective, pathogenic bacteria

[0077] In a healthy animal, the internal tissues, *e.g.* blood, brain, muscle, *etc.*, are normally free of microorganisms. On the other hand, the surface tissues, *e.g.*, skin and mucous membranes, are constantly in contact with environmental organisms and become readily colonized by certain microbial species. The normal flora is the mixture of organisms regularly found at an anatomical site, *e.g.*, skin, conjunctiva, nose, pharynx, mouth, lower intestine, anterior urethra, and vagina.

[0078] The normal flora of humans, *e.g.*, is exceedingly complex and consists of more than 200 species of bacteria. Clinical microbiology studies these and pathogenic strains, and other aspects of the related biology relevant to human health. *See, e.g.*, Sarma *Medical Microbiology: A Clinical Perspective* Paras, Heyderabad, ISBN: 8188129070 (2001); Baron, *et al. Bailey & Scott's Diagnostic Microbiology* (9th ed.), ISBN: 0801669871 (1994); Balows, *et al.* (eds. 1991) *Manual of Clinical Microbiology* (5th ed.) Am. Soc. Microbiol., Wash. D.C., ASIN: 1555810306; Hobbs, *et al. Medical Microbiology for Students*, Arnold, New Delhi (1991); and Fessia, *et al. Diagnostic Clinical Microbiology: A Benchtop Perspective*, Saunders, Philadelphia, ISBN: 0721623263 (1988). The makeup of the normal flora depends upon various factors, including genetics, age, sex, stress, nutrition, and diet of

the individual. The normal flora of humans includes eukaryotic fungi and protists, and some methanogenic Archaea that colonize the lower intestinal tract, but bacteria are the most numerous and obvious microbial components of the normal flora.

[0079] The normal flora are obviously adapted to their host (tissues), most probably by biochemical interactions between bacterial surface components (ligands or adhesins) and host cell molecular receptors. Much information is available on the nature of adhesion of bacterial pathogens to animal cells and tissues, and reasonably similar mechanisms should apply to the normal flora.

[0080] Little is known about the nature of the associations between humans and their normal flora, but they are thought to be dynamic interactions rather than associations of mutual indifference. Both host and bacteria are thought to derive benefit from each other, and the associations are, for the most part, mutualistic. The normal flora derives from the host a supply of nutrients, a stable environment, and constant temperature, protection, and transport. The host obtains from the normal flora certain nutritional benefits, stimulation of the immune system, and colonization strategies that exclude potential pathogens at the site.

[0081] A pathogenic microorganism causes disease. Pathogenicity is the ability to produce disease in a host organism. Microbes express their pathogenicity by means of their virulence, a term which refers to the degree of pathogenicity of the microbe. Hence, the determinants of virulence of a pathogen are its genetic or biochemical or structural features that enable it to produce disease in a host.

[0082] The relationship between a host and a pathogen is dynamic, since each modifies the activities and functions of the other. The outcome of an infection depends on the virulence of the pathogen and the relative degree of resistance or susceptibility of the host, due mainly to the effectiveness of the host defense mechanisms.

[0083] Historically, bacteria have been the cause of some of the most deadly diseases and widespread epidemics of human civilization. *See, e.g.*, Cohen *Infectious Diseases*, Elsevier, ISBN: 0323024076 (2003); Gorbach, *et al. Infectious Diseases* Lippincott Williams & Wilkins, ISBN: 0781733715 (eds. 2003); Turkington, *et al. Encyclopedia of Infectious Diseases* (2d ed.) Facts on File, ISBN: 0816047758 (2003); Watstein and Jovanovic *Statistical Handbook on Infectious Diseases* Greenwood, ISBN: 1573563757 (2003); Mandell *Principles and Practice of Infectious Diseases* Elsevier, ISBN: 0443065810 (2000); Gorbach and Stone *Atlas of Infectious Diseases*, Harcourt, ISBN: 0721670326 (2000); Root,

et al. Clinical Infectious Diseases: A Practical Approach, Oxford University Press, ISBN: 0195143493 (eds. 2000); Schlossberg *Current Therapy of Infectious Disease* (2d ed.) Elsevier, ISBN: 0323009077 (2000); and Mandell, *et al. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*, Churchill Livingstone, ISBN: 044307593X (1998).

[0084] Public health measures, *e.g.*, water purification, immunization, and modern antibiotic treatment, have reduced the morbidity and the mortality of bacterial disease in the Twentieth Century, at least in the developed world where these are acceptable cultural practices. However, many new bacterial pathogens have been recognized in the past 25 years and many "old" bacterial pathogens, such as *Staphylococcus aureus* and *Mycobacterium tuberculosis*, have emerged with new determinants of virulence as well as new patterns of resistance to antimicrobial agents.

B. Nosocomial (hospital derived) infections, environmental bacteria, and pyogenic (pus forming) bacteria

[0085] The methods and compositions of the invention can be used to inhibit growth of nosocomial bacteria, including bacteria that populate the hospital environment, or bacteria that are present on human skin, or bacteria that infect and form pus in wounds,. Nosocomial infections are infections which become evident during a hospital stay or are related to a procedure performed in a hospital. The procedure-related infections often become evident after patients are discharged from the hospital. The most common nosocomial infections are urinary tract infections, surgical-site infections, pneumonia, and serious systemic infections, in which bacteria or fungi can be grown from blood.

[0086] Acquiring a microbe in a hospital does not cause a nosocomial infection itself. It is often stated that a patient 'contracted' a hospital bug and the surgical wound was infected. However, the issue is more complex.

[0087] The development of a nosocomial infection is a chain of events, which is influenced by the microbe, transmission route, and patient him/herself, *i.e.*, his/her underlying illness, resistance to infections, and treatment of the underlying illness. Most nosocomial infections are caused by microbes which are present in the microbial flora on the skin or mucous membranes of the patient. To a lesser extent, microbes originate from outside the body: another patient, staff, or hospital environment. In addition, the microbial flora of the patient often change during the hospital stay, mostly due to antimicrobial treatment. Modern

treatments often necessitate the use of intravenous catheters, urinary catheters, respirators, hemodialysis, complicated operations, cortisone therapy and other factors, which depress resistance mechanisms and make patients susceptible to infections.

[0088] Institutional patients frequently develop nosocomial infections that are caused by normal flora colonizing the patient at the time of admission, or by exogenous pathogens that are acquired and subsequently colonize the patient after admission, *e.g.*, to the hospital. A variety of strategies have been used either to prevent colonization from occurring, to eradicate colonizing organisms, or to prevent the progression from colonization to infection. These strategies include implementation of infection control measures designed to prevent acquisition of exogenous pathogens, eradication of exogenous pathogens from patients or personnel who have become colonized, suppression of normal flora, prevention of colonizing flora from entering sterile body sites during invasive procedures, microbial interference therapy, immunization of high-risk patients, and modification of antibiotic utilization practices. Strategies that require widespread use of antimicrobial agents to suppress or eradicate colonizing organisms tend to promote emergence of multidrug-resistant pathogens, Thus, a large number of potential infectious diseases lurk in environments where treatment is provided.

[0089] The methods and compositions of the invention are used to inhibit growth of gram negative or gram positive bacteria. Gram positive bacteria include *e.g.*, *Staphylococcus* (pyogenic) and *Enterococcus* (opportunistic). Gram negative bacteria include *e.g.*, *Pseudomonas* (pyogenic), *E. coli* (opportunistic), *Salmonella* (opportunistic), *Campylobacter* (opportunistic), *Proteus* (pyogenic), *Klebsiella* (opportunistic), *Enterobacter* (pyogenic), and *Citrobacter* (pyogenic). The pyogenic cocci are spherical bacteria that cause various suppurative (pus-producing) infections in animals. Included are the Gram-positive cocci *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*, and the Gram-negative cocci, *Neisseria gonorrhoeae*, and *N. meningitidis*. In terms of their phylogeny, physiology, and genetics, these genera of bacteria are unrelated to one another.

[0090] The Gram-positive cocci are the leading pathogens of humans. It is estimated that they produce at least a third of all the bacterial infections of humans, including strep throat, pneumonia, food poisoning, various skin diseases, and severe types of septic shock. The Gram-negative cocci, notably the *Neisseriae*, cause gonorrhea and meningicoccal meningitis.

C. Antibiotic resistant bacteria

[0091] The methods and compositions of the invention are used to inhibit growth, particularly of antibiotic resistant bacteria. For example, numerous bacterial pathogens of great importance to mankind have become multi-drug resistant (MDR), and these MDR strains have spread rapidly around the world. As a result, hundreds of thousands of people now die each year from infections that could have been successfully treated by antibiotics just 4-5 years ago. *See, e.g.*, Kunin *Annals of Internal Medicine* 118:557-561 (1993); and Neu *Science* 257:1064-73 (1992). In the case of MDR tuberculosis, *e.g.*, immunocompromised as well as non-immunocompromised patients in our era are dying within the first month or so after the onset of symptoms, despite the use of as many as 11 different antibiotics.

[0092] Medical authorities have described multi-drug resistance not just for TB, but for a wide variety of other infections as well. Some infectious disease experts have termed this situation a "global crisis". In fact, efforts at developing new antibiotics are rather limited. A search is underway for alternative modes and novel mechanisms for treating these MDR bacterial infections.

[0093] Genetic variability in bacteria may also be created by acquisition of foreign DNA carried by plasmids, bacteriophages, or transposable genetic elements. An example of this phenomenon is the spread of a tetracycline-resistant transposon among *Neisseria gonorrhoeae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. These mechanisms allow bacteria the potential to develop resistance to a conventional antibiotic. *See* Beers and Borkow *The Merck Manual* (17th ed.) Merck (eds. 2003).

D. Diagnosis of bacterial infection

[0094] The diagnosis of bacterial infections assists in understanding the basis of infectious disease pathological symptoms. In particular, the detection and characterization of the local flora can be useful to determine the components and effects attributable to presence of infectious diseases. The genetic composition of the various strains and the interactions between strains and the host contribute to the resulting microbiological environment.

[0095] Initial diagnosis of potential or actual infectious agents, *e.g.*, bacteria, typically leads to treatment strategies and methods. Thus, the ability to diagnose a bacterial infection can be used to identify the causative agent and treatment methods which can be appropriate to the specific infection. Methods of diagnosing bacterial infections are know to those of skill in the art, *see, e.g.*, MacFaddin *Biochemical Tests for Identification of Medical Bacteria*

(3d ed.) Lippincott, Williams & Wilkins, ISBN: 0683053183 (2000); and Balows and Balows *Biotyping in the Clinical Microbiology Laboratory* Thomas Pubs (1978).

[0096] The present invention provides methods to diagnose bacterial infections using anti-bacterial phages. The method is based, in large part, on specific interactions between an anti-bacterial phage and a target bacterium. Those of skill will recognize method to label anti-bacterial phages and to use labeled anti-bacterial phages to detect a target bacterium in a biological sample from a subject suspected of having a bacterial infection.

IV. ANTI-BACTERIAL PHAGE

[0097] Anti-bacterial phages of the present invention are useful to treat bacterial infections in a target bacterium. In particular, because the anti-bacterial phages are unable to replicate in the target bacterium, the anti-bacterial phages can be administered in a defined dose.

[0098] Anti-bacterial phages are also particularly useful as anti-bacterial agents in an environment where bacterial growth is not desired or is considered to be harmful. For example, anti-bacterial phage preparations can be used to sterilize medical settings, *e.g.* operating room suites or food preparation areas, particularly areas where raw meat, *e.g.*, beef, lamb, poultry, pork, or fish is handled.

A. Methods to diminish replication activity of anti-bacterial phages in a target bacterium

[0099] Non-replicating phage constructs can be generated by making intact phage, and removing or inactivating the genetic material. Methods for removing the nucleic acid include, *e.g.*, osmotic shock, freeze thaw, chemical treatment, or mechanical removal. Such may allow the nucleic acid to escape. The phage coat may reassemble and reseal, or the DNA containing head segment of a phage may be lost. In many cases, the attaching and killing functions of the fragmented phage will be retained, while the genetic capacity of the composition is absent.

[0100] Osmotic shock of phage may be performed, *e.g.*, with salts or sugars. Freeze-thaw cycles of phage may result in mechanical or other fragmentation which allows for functional separation of the attaching/killing functions (*e.g.*, provided by phage tails) and the genetic replication function. Chemical treatments of phage have also been observed to fragment the phage, *e.g.*, treatment with LiCl or other salts; EDTA and/or other chelating agents; organic salts; amino acids; and reducing agents. Mechanical methods of fragmenting phage are

available, including, *e.g.*, shearing, lyophilization, sonication, microwave treatment, and others.

[0101] Another method of inactivating phage nucleic acid, *e.g.*, UV irradiation, DNA fragmentation, chemical destruction (*e.g.*, by D-glucosamine treatment), or ferrous ammonium sulfate. DNA modifying reagents can destroy the functional capacity of nucleic acids in phage, by preventing replication of the nucleic acid itself, by preventing assembly of an intact phage, by preventing release of phage from an infected bacterium, or by preventing the replication of genetically competent phage. Methods of chemical destruction of phage nucleic acids are found in the following references: Watanabe, K. *et al.*,. *Agric. Biol. Chem.* 49 (1), 63-70 (1985); Kashige, N., *et al.*, *Carbohydr. Res.* 257 (2), 285- 291 (1994); Kakita, Y., *et al.*, *Microbiol. Immunol.* 39 (8), 571-576 (1995).; Yamaguchi, T., *et al.*, Biol. Pharm. Bull. 19 (10), 1261-1265 (1996); Kakita, Y., *et al.*, *Biosci. Biotechnol. Biochem.* 61 (11), 1947-1948 (1997); Yamaguchi, T., *et al*, *Biol. Pharm. Bull.* 21 (3), 205-209 (1998); Yamaguchi, T.,*et al.*, *Tetrahedron* 55 (3), 675-686 (1999); Watanabe, K., *et al.*, *Lett. Appl. Microbiol.* 31 (1), 52-56 (2000); Kashige, N., *et al.*, *Biol. Pharm. Bull.* 23 (11), 1281-1286 (2000); and Kashige, N., *et al.*, *Curr. Microbiol.* 42 (3), 184-189 (2001).

[0102] In another embodiment, a replication incompetent phage lacks detectable nucleic acid component entirely. Such include partial phage, *e.g.*, which lack the nucleic acids or the structural compartments which contain the nucleic acid component. These have been referred to as "ghosts" in certain studies on phage structure and the components functionally required to achieve infection processes. There are various methods for generating these constructs. Intact phage may be fragmented, and the tail portions which are involved in the binding and killing of target bacteria are often retained. The phage particles may be harvested from their infective cycles before the phage are completely assembled according to the genetic program for production and assembly of the phage within the bacterial host. The phage can be harvested after tail assembly before attachment of the heads which contain genetic material.

[0103] Other replication incompetent phage can have disabled or incomplete phage genomes, *e.g.*, prophages. Such phage may be intact, but lack critical parts of the genome, *e.g.*, critical replication or assembly proteins. Simple embodiments include phage with genetic lesions or insertions in one or more critical genes. More complex embodiments include phage with termination codons in critical genes, which prevent expression or function

of the gene products. Significant genetic deletions are also available, for which reversion mutations should be extremely rare. However, many of the genetically deficient phage may need to be produced with helper phage, or special complementing production host systems to provide the mutated faunction. These production host systems can be made by transforming them with genes encoding the deficiencies in the phage, *e.g.*, to complement the deficiencies in those hosts. However, means to prevent genetic transfer from the host to the phage would be desired.

[0104] Thus, specific production host strains may be important in production of specific phage. For example, critical assembly genes may be deleted from the phage but provided by the production hosts, and, in these embodiments, the phage are capable of being produced only in such host. Helper phage are another strategy to complement the deficiency, but means to prevent genetic recombination into the phage genome would be needed. Examples of phage mutations and complementing host or phage include phage comprising termination mutations and complementing host or phage comprising tRNA suppressors, phage comprising mutations in genes critical for replication and complementing host or phage comprising antisense constructs that complement the mutation, phage comprising insertion mutations and complementing host or phage that comprise suppressors of the mutations, and phage comprising deletion mutations and complementing host or phage that comprise suppressors of the mutations.

[0105] Certain phage fragments can be assembled in vitro from purified protein components and used as anti-bacterial phage compounds.

[0106] For tailed phage, separation of nucleic acid-containing phage heads from phage tails can be performed to produce anti-bacterial phages, *i.e.*, the isolated tails. Those of skill will recognize that phage head and tails can be separated using a variety of techniques based on physical properties of the phage heads and tails, *e.g.*, separation by size, by charge or by other properties. For example, only phage heads contain nucleic acids and this can be exploited in separation by *e.g.*, gradient centrifugation. Other separation techniques, based largely on protein purification techniques follow.

Solubility fractionation

[0107] Often as an initial step, an initial salt fractionation can separate many of the unwanted phage components (or proteins derived from the cell culture media) from the anti-bacterial phage tails The preferred salt is ammonium sulfate. Ammonium sulfate precipitates

proteins and protein complexes by effectively reducing the amount of water in the protein mixture. Proteins and protein complexes then precipitate on the basis of their solubility. The more hydrophobic a protein is, the more likely it is to precipitate at lower ammonium sulfate concentrations. A typical protocol includes adding saturated ammonium sulfate to a protein solution so that the resultant ammonium sulfate concentration is between 20-30%. This concentration will precipitate the most hydrophobic of proteins. The precipitate is then discarded (unless the protein of interest is hydrophobic) and ammonium sulfate is added to the supernatant to a concentration known to precipitate the protein of interest. The precipitate is then solubilized in buffer and the excess salt removed if necessary, either through dialysis or diafiltration. Other methods that rely on solubility of proteins, such as cold ethanol precipitation, are well known to those of skill in the art and can be used to fractionate complex protein mixtures.

Size differential filtration

[0108] The molecular weight of the anti-bacterial phage, *e.g.*, the phage tail, can be used to isolate it from phage components of greater and lesser size using ultrafiltration through membranes of different pore size (for example, Amicon or Millipore membranes). As a first step, the phage mixture is ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then ultrafiltered against a membrane with a molecular cut off greater than the molecular weight of the protein of interest. The recombinant protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed as described below.

Column chromatography

[0109] The phage componentss can also be separated from each other on the basis of its size, net surface charge, hydrophobicity, and affinity for ligands. In addition, antibodies raised against phage components can be conjugated to column matrices and the proteins immunopurified. All of these methods are well known in the art. It will be apparent to one of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech).

C. Therapeutic treatment using anti-bacterial phages

[0110] The present invention can be applied across the spectrum of bacterial diseases, so that phage derived compositions are developed that are specific for each of the bacterial

strains of interest. *See, e.g.*, Merril, *et al.*, Pat. App. US 2003/0026785 and Loomis and Fischetti, Pat. App. US 2002/0187136, each of which are herein incorporated by reference for all purposes. In that way, a full array of compositions is developed for virtually all the bacterial (and other applicable) pathogens for man, his pets, livestock and zoo animals (whether mammal, avian, or pisciculture). Phage derived therapy will then be available, *e.g*, 1) as an adjunct to or as a replacement for those antibiotics and/or chemotherapeutic drugs that are no longer functioning in a bacteriostatic or bactericidal manner due to the development of multi-drug resistance; 2) as a treatment for those patients who are allergic to the antibiotics and/or chemotherapeutic drugs that would otherwise be indicated; and 3) as a treatment that has fewer side effects than many of the antibiotics and/or chemotherapeutic drugs that would otherwise be indicated for a given infection.

[0111] A second embodiment of the present invention is the development of methods to treat bacterial infections in animals through phage derived therapy with the compositions described above. Hundreds of bacteriophages and the bacterial species they infect are known in the art. The present invention is not limited to a specific bacteriophage or a specific bacteria. Rather, the present invention can be utilized to develop bacteriophage derived compositions which can be used to treat many infections caused by their host bacteria.

[0112] While it is contemplated that the present invention can be used to treat most bacterial infections in an animal, it is particularly contemplated that the methods described herein will be very useful as a therapy (adjunctive or stand-alone) in infections caused by drug-resistant bacteria. Experts report (*See, e.g.*, Gibbons *Science*, Aug. 21, 1993, pp. 1036-38 (1993)) drug-resistant bacterial species and strains which represent the greatest threats to mankind. *See, e.g.*, Merril, *et al.*, Pat. App. US 2003/0026785, pages 4-5; and Loomis and Fischetti, Pat. App. US 2002/0187136 page 5. These include, *e.g.*, the clinically important members of the family *Enterobacteriaceae*, most notably, but not limited to the clinically important strains of *Escherichia* (most notably *E. coli*); *Klebsiella* (most notably *K. pneumoniae*); *Shigella* (most notably *S. dysenteriae*); *Salmonella* (including *S. abortus-equi, S. typhi, S. typhimurium, S. newport, S. paratyphi-A, S. paratyphi-B, S. potsdam*, and *S. pollurum*); *Serratia* (most notably *S. marcescens)*; *Yersinia* (most notably *Y. pestis*); and *Enterobacter* (most notably *E. cloacae*). Other important groups include *Enterococci*, most notably *E. faecalis* and *E. faecium*; *Haemophilus*, most notably *H. influenzae*; *Mycobacteria*, most notably *M. tuberculosis, M. avium-intracellulare, M. bovis*, and *M. leprae; Neisseria gonorrhoeae* and *N. meningitidis; Pseudomonads*, most notably *P. aeuruginosa*;

Staphylococci, most notably *S. aureus* and *S. epidermidis*; *Streptococci*, most notably *S. pneumoniae*; and *Vibrio cholera*.

[0113] There are additional bacterial pathogens too numerous to mention that, while not currently in the state of antibiotic-resistance crisis, nevertheless make excellent candidates for treatment with these compositions, in accordance with the present invention. Thus, bacterial infections caused by bacteria for which there is a corresponding phage can often be treated using the present invention. *See, e.g.*, Loomis and Fischetti, Pat. App. US 2002/0187136, page 5.

[0114] A phage strain capable of doing direct or indirect harm to a bacteria (or other pathogen) is contemplated as useful in the present invention. Thus, phages that are lytic, phages that are temperate but can later become lytic, and nonlytic phages that can deliver a product that will be harmful to the bacteria are all useful in the present invention.

[0115] Animals to be treated by the methods of the present invention include but are not limited to man, his domestic pets, livestock, pisciculture, and the animals in zoos and aquatic parks (such as whales and dolphins). Anti-bacterial phage can also by used to treat bacterial infections in plants.

[0116] The compositions of the present invention can be used as a stand-alone therapy or as an adjunctive therapy for the treatment of bacterial infections. Numerous antimicrobial agents (including antibiotics and chemotherapeutic agents) are known which would be useful in combination with these compositions for treating bacterial infections. Examples of suitable antimicrobial agents and the bacterial infections which can be treated with the specified antimicrobial agents are known. *See, e.g.*, Merril, *et al.*, Pat. App. US 2003/0026785, page 5. However, the present invention is not limited to the antimicrobial agents listed, as one skilled in the art could easily determine other antimicrobial agents useful in combination with these compositions.

D. Methods to identify anti-bacterial phages

[0117] As a first step, a method to identify an anti-bacterial phage will begin by identifying a target bacterium. Methods to identify a phage that infects a target bacterium, *e.g.*, a wild-type, naturally occurring phage, are known to those of skill in the art. The methods described herein can be used to produce a form of the wild-type naturally occurring phage that kills the target bacterium, but lacks replication activity in the target bacterium, *i.e.*, an anti-bacterial phage.

V. ADMINISTRATION

[0118] The route of administration and dosage will vary with the infecting bacteria, the site and extent of infection (*e.g.*, local or systemic), and the subject being treated. The routes of administration include but are not limited to: oral, aerosol or other device for delivery to the lungs, nasal spray, intravenous (IV), intramuscular, intraperitoneal, intrathecal, vaginal, rectal, topical, lumbar puncture, intrathecal, and direct application to the brain and/or meninges. Excipients which can be used as a vehicle for the delivery of the phage will be apparent to those skilled in the art. For example, the free phage could be in lyophilized form and be dissolved just prior to administration by IV injection. The dosage of administration is contemplated to be in the range of about 1million to about 10 trillion/per kg/per day, and preferably about 1 trillion/per kg/per day, and may be from about 10^6 killing units/kg/day to about 10^{13} killing units/kg/day.

[0119] Methods to evaluate killing capacity are similar to methods used by those of skill to evaluate intact replicating phage, *i.e.*, plaque forming units or pfu. Killing quantitation is more distinct, however, since the non-replicating phage will not form plaques on bacterial host lawns. Thus, serial dilution methods to evaluate the quantity of "killing" units are conveniently used in place of standard pfu. The particular method used to establish killing units is not critical to the invention. Serial dilutions of bacterial cultures exposed to the killing compositions can quantitate killing units. Alternatively, comparing total bacterial counts with viable colony units can establish what fraction of bacteria are actually viable, and by implication, what fraction have been susceptible to the killing constructs.

[0120] The phage are administered until successful elimination of the pathogenic bacteria is achieved. Thus the invention contemplates single dosage forms, as well as multiple dosage forms of the compositions of the invention, as well as methods fro accomplishing delivery of such single and multi-dosages forms.

[0121] With respect to the aerosol administration to the lungs, the phage composition is incorporated into an aerosol formulation specifically designed for administration to the lungs by inhalation. Many such aerosols are known in the art, and the present invention is not limited to any particular formulation. An example of such an aerosol is the Proventil™ inhaler manufactured by Schering-Plough, the propellant of which contains trichloromonofluoromethane, dichlorodifluoromethane and oleic acid. The concentrations of the propellant ingredients and emulsifiers are adjusted if necessary based on the phage

composition being used in the treatment. The number of phage to be administered per aerosol treatment will be in the range of 10^6 to 10^{13} killing units, and preferably 10^{12} killing units.

[0122] Methods to evaluate killing capacity are similar to many methods used in working with intact replicating phage. In particular, killing quantitation is more difficult since the non-replicating phage will not form plaques on bacteria. Thus, serial dilution methods to evaluate the quantity of "killing" units will be performed similarly to standard pfu (plaque forming units), but cannot make use of the killing and amplification which occurs on a bacterial host lawn. Serial dilutions of bacterial cultures exposed to the killing compositions can quantitate killing units. Alternatively, comparing total bacterial counts with viable colony units can establish what fraction of bacteria are actually viable, and by implication, what fraction have been susceptible to the killing constructs.

[0123] Methods to evaluate the replication capacity of a construct can use normal plaque forming assays. Typically, the inactivation will decrease the replication capacity by at least 3 fold, and may affect it by 10, 30, 100, 300, etc., to many orders of magnitude. Preferred genetic inactivation efficiencies may be 4, 5, 6, 7, 8, or more log units.

VI. FORMULATIONS

[0124] The invention further contemplates pharmaceutical compositions comprising at least one bacteriophage of the invention provided in a pharmaceutically acceptable excipient. The formulations and pharmaceutical compositions of the invention thus contemplate formulations comprising an isolated bacteriophage specific for a bacterial host; a mixture of two, three, five, ten, or twenty or more bacteriophage that infect the same bacterial host; and a mixture of two, three, five, ten, or twenty or more bacteriophage that infect different bacterial hosts or different strains of the same bacterial host. (*e.g.*, a mixture of bacteriophage that collectively infect and inhibit the growth of multiple strains of Staphylococcus aureus). In this manner, the compositions of the invention can be tailored to the needs of the patient.

[0125] By "therapeutically effective dose" herein is meant a dose that produces effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*e.g.*, Ansel *et al.*, *Pharmaceutical Dosage Forms and Drug Delivery*; Lieberman, Pharmaceutical Dosage Forms (vols. 1-3, 1992), Dekker, ISBN 0824770846, 082476918X, 0824712692, 0824716981; Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding*

(1999); and Pickar, *Dosage Calculations* (1999)). As is known in the art, adjustments for protein degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

[0126] Various pharmaceutically acceptable excipients are well known in the art. As used herein, "pharmaceutically acceptable excipient" includes a material which, when combined with an active ingredient of a composition, allows the ingredient to retain biological activity and without causing disruptive reactions with the subject's immune system.

[0127] Exemplary pharmaceutically carriers include sterile aqueous of non-aqueous solutions, suspensions, and emulsions. Examples include, but are not limited to, standard pharmaceutical excipients such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/ aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like.

[0128] A composition comprising a bacteriophage of the invention may also be lyophilized using means well known in the art, for subsequent reconstitution and use according to the invention.

[0129] Also of interest are formulations for liposomal delivery, and formulations comprising microencapsulated bacteriophage. Compositions comprising such excipients are formulated by well known conventional methods (*see*, for example, *Remington's Pharmaceutical Sciences*, Chapter 43, 14th Ed., Mack Publishing Col, Easton PA 18042, USA).

[0130] In general, the pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules (*e.g.* adapted for oral delivery) , microbeads, microspheres, liposomes, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions comprising the therapeutically-active compounds. Diluents known to

the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value.

[0131] The pharmaceutical composition can comprise other components in addition to the bacteriophage. In addition, the pharmaceutical compositions may comprise more than one bacteriophage, for example, two or more, three or more, five or more, or ten or more different bacteriophage, where the different bacteriophage may be specific for the same or different bacteria. For example, the pharmaceutical composition can contain multiple (*e.g.*, at least two or more) defined holin-modified bacteriophage, wherein are least two of the phage in the composition have different bacterial host specificity. In this manner, the holin-modified bacteriophage composition can be adapted for treating a mixed infection of different bacteria, *e.g.*, by selecting different groups of bacteriophage of differing specificity so as to contain at least one bacteriophage for each bacteria (*e.g.*, strain, species, etc.) suspected of being present in the infection (*e.g*, in the infected site). As noted above, the bacteriophage can be administered in conjunction with other agents, such as a conventional antimicrobial agent (see table above). In some embodiments, it may be desirable to administer the bacteriophage and antibiotic within the same formulation.

VII. METHODOLOGY

[0132] Some aspects of practicing the present invention involve well-known methods general clinical microbiology, general methods for handling bacteriophage, and general fundamentals of biotechnology, principles and methods. References for such methods are listed below and are herein incorporated by reference for all purposes.

A. General clinical microbiology

[0133] General microbiology is the study of the microorganisms. *See, e.g.*, Sonenshein, *et al. Bacillus Subtilis and Its Closest Relatives: From Genes to Cells* Amer. Soc. Microbiol., ISBN: 1555812058 (ed. 2002); Alexander and Strete *Microbiology: A Photographic Atlas for the Laboratory* Benjamin/Cummings, ISBN: 0805327320(2001); Cann *Principles of Molecular Virology* (Book with CD-ROM; 3d ed.), ISBN: 0121585336 (2001); Garrity *Bergey's Manual of Systematic Bacteriology Volume 1: The Archaea, Cyanobacteria, Phototrophs & Deeply* (2d ed.) Springer Verlag, ISBN: 0387987711 (ed. 2001); Salyers and Whitt *Bacterial Pathogenesis: A Molecular Approach* (2d ed.) Amer. Soc. Microbiol., ISBN: 155581171X (2001); Tierno *The Secret Life of Germs: Observations and Lessons from a*

Microbe Hunter Pocket Star, ISBN: 0743421876 (2001); Block *Disinfection, Sterilization, and Preservation* (5th ed.) Lippincott Williams & Wilkins Publ., ISBN: 0683307401 (ed. 2000); Cullimore *Practical Atlas for Bacterial Identification* Lewis Pub., ISBN: 1566703921 (2000); Madigan, *et al. Brock Biology of Microorganisms* (9th ed.) Prentice Hall, ASIN: 0130819220 (2000); Maier, *et al. Environmental Microbiology* Academic Pr., ISBN: 0124975704 (eds. 2000); Tortora, *et al. Microbiology: An Introduction* including Microbiology Place(TM) Website, Student Tutorial CD-ROM, and Bacteria ID CD-ROM (7th ed.), Benjamin/Cummings, ISBN 0805375546 (2000); Demain, *et al. Manual of Industrial Microbiology and Biotechnology* (2d ed.) Amer. Soc. Microbiol., ISBN: 1555811280 (eds. 1999); Flint, *et al. Principles of Virology: Molecular Biology, Pathogenesis, and Control* Amer. Soc. Microbiol., ISBN: 1555811272 (eds. 1999); Murray, *et al. Manual of Clinical Microbiology* (7th ed.) Amer. Soc. Microbiol., ISBN: 1555811264 (ed. 1999); Burlage, *et al. Techniques in Microbial Ecology* Oxford Univ. Pr., ISBN: 0195092236 (eds. 1998); Forbes, *et al. Bailey & Scott's Diagnostic Microbiology* (10th ed.) Mosby, ASIN: 0815125356 (1998); Schaechter, *et al. Mechanisms of Microbial Disease* (3d ed.) Lippincott, Williams & Wilkins, ISBN: 0683076051 (ed. 1998); Tomes *The Gospel of Germs: Men, Women, and the Microbe in American Life* Harvard Univ. Pr., ISBN: 0674357078 (1998); Snyder and Champness *Molecular Genetics of Bacteria* Amer. Soc. Microbiol., ISBN: 1555811027 (1997); Karlen *MAN AND MICROBES: Disease and Plagues in History and Modern Times* Touchstone Books, ISBN: 0684822709 (1996); and Bergey *Bergey's Manual of Determinative Bacteriology* (9th ed.) Lippincott, Williams & Wilkins, ISBN: 0683006037 (ed. 1994).

B. General methods for handling bacteriophage

[0134] General methods for handling bacteriophage are well known, *see, e.g.*, Snustad and Dean *Genetics Experiments with Bacterial Viruses* Freeman (2002); O'Brien and Aitken Antibody Phage Display: Methods and Protocols Humana (eds. 2002); Ring and Blair *Genetically Engineered Viruses* BIOS Sci. Pub. (eds. 2000); Adolf *Methods in Molecular Genetics: Viral Gene Techniques* vol. 6, Elsevier (ed. 1995); Adolf *Methods in Molecular Genetics: Viral Gene Techniques* vol. 7, Elsevier (ed. 1995); and Hoban and Rott *Molec. Biol. of Bacterial Virus Systems* (Current Topics in Microbiology and Immunology No. 136) Springer-Verlag (eds. 1988).

C. General fundamentals of biotechnology, principles and methods

[0135] General fundamentals of biotechnology, principles and methods are described, *e.g,* in Alberts, *et al. Molecular Biology of the Cell* (4th ed.) Garland ISBN: 0815332181 (2002); Lodish, *et al. Molecular Cell Biology* (4th ed.) Freeman, ISBN: 071673706X (1999); Janeway, *et al. Immunobiology* (5th ed.) Garland, ISBN: 081533642X (eds. 2001); Flint, *et al. Principles of Virology: Molecular Biology, Pathogenesis, and Control*, Am. Soc. Microbiol., ISBN: 1555811272 (eds. 1999); Nelson, *et al. Lehninger Principles of Biochemistry*, (3d ed.) Worth, ISBN: 1572599316 (2000); Freshney *Culture of Animal Cells: A Manual of Basic Technique* (4th ed.) Wiley-Liss; ISBN: 0471348899 (2000); Arias and Stewart *Molecular Principles of Animal Development*, Oxford University Press, ISBN: 0198792840 (2002); Griffiths, *et al. An Introduction to Genetic Analysis* (7th ed.) Freeman, ISBN: 071673771X (2000); Kierszenbaum *Histology and Cell Biology*, Mosby, ISBN: 0323016391 (2001); Weaver *Molecular Biology* (2d ed.) McGraw-Hill, ISBN: 0072345179 (2001); Barker *At the Bench: A Laboratory Navigator CSH Laboratory*, ISBN: 0879695234 (1998); Branden and Tooze *Introduction to Protein Structure* (2d ed.), Garland Publishing; ISBN: 0815323050 (1999); Sambrook and Russell *Molecular Cloning: A Laboratory Manual* (3 vol., 3d ed.), CSH Lab. Press, ISBN: 0879695773 (2001); and Scopes *Protein Purification: Principles and Practice* (3d ed.) Springer Verlag, ISBN: 0387940723 (1994).

[0136] It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a bacteriophage" includes a plurality of such bacteriophage and reference to "the host bacterium" includes reference to one or more host bacteria and equivalents thereof known to those skilled in the art, and so forth.

[0137] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed. Citations are incorporated herein by reference.

[0138] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0139] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims

EXAMPLES

Example 1: Methods of inactivating or removing nucleic acid to make anti-bacterial phage.

a. Osmotic shock treatment

[0140] Nucleic acids can be released from phage upon osmotic shock treatment. Phage are prepared and subjected to osmotic shock at an appropriate temperature, *e.g.*, low temperature, and for an appropriate amount of time, *e.g.*, 1-60 minutes, depending upon the phage type and strain. *See, e.g.*, Minagawa *Virology* 76:234-245 (1977) (NaCl shock) or Szewczyk and Skorko *Biochim. Biophys. Acta* 662:131-137 (1981) (sucrose shock). Other osmotic agents can be used, and the shock medium may be supplemented with, *e.g.*, appropriate amounts of nucleases, proteases, protease inhibitors, etc.

[0141] After the removal of the nucleic acid, the intact, replication competent phage are removed from the preparation. Such can be achieved, *e.g.*, by size or weight based separation methods. A preferred method is density separation, as the phage particles lacking nucleic acid differentially separate from intact particles. The inactivated phage, *i.e.*, anti-bacterial phage, are collected, and the intact phage my be collected or discarded. Intact phage may be useful as starting materials for a second treatment, or for diagnostic or other uses where the replication capacity may be useful.

b. EDTA treatment

[0142] Phage subjected to EDTA treatment yield DNA deficient phage. EDTA treated phage retain target bacterium binding capacity, and kill target bacteria. *See, e.g.*, Konopa and Taylor *Biochim. Biophys. Acta* 399:460-467 (1975). The treated phage are subjected to purification methods to separate intact, replication competent phage from anti-bacterial phage that lack nucleic acid using *e.g.*, density separation.

c. Amino acid treatment

[0143] Exposure to various amino acids have been reported to affect phage replication competence. See Murata, *et al. Agr. Biol. Chem.* 38:477-478 (1974). Various amino acids may be used, and the time and conditions to which the phage are exposed are optimized for

the specific phage and target host pair. The treated phage are subjected to purification methods to separate intact, replication competent phage from anti-bacterial phage that lack nucleic acid using *e.g.*, density separation.

d. Lyopholization treatment

[0144] Phage subjected to lyophilization become replication deficient, while retaining target bacterium killing capacity. *See, e.g.*, Shapira and Kohn *Cryobiology* 11:452-464 (1974); and Clark and Geary *Cryobiology* 10:351-360 (1973). The treated phage are subjected to purification methods to separate intact, replication competent phage from anti-bacterial phage that lack nucleic acid using *e.g.*, density separation.

e. Microwave treatment

[0145] Exposure of bacteriophage to microwave irradiation can diminish the replication capacity of a phage. *See, e.g.*, Kikita, et al. *Microbiol. Immunol.* 39:571-576 (1995); and Watanabe, *et al. Lett. Appl. Microbiol.* 31:52-56 (2000). The treated phage are subjected to purification methods to separate intact, replication competent phage from anti-bacterial phage that lack nucleic acid using *e.g.*, density separation.

f. UV or X-ray irradiation treatment

[0146] Irradiation by UV or X-rays inactivates the replication capacity of phage. The treated phage are subjected to purification methods to separate intact, replication competent phage from anti-bacterial phage that lack nucleic acid using *e.g.*, density separation.

g. D-glucosamine treatment

[0147] D-glucosamine treatment of phage inactivates the replication competence of phage, while retaining the killing capacity. *See, e.g.*, Watanabe, *et al. Agr. Biol. Chem.* 49:63-70 (1985); and Yamaguchi, *et al. Biol. Pharm. Bull.* 21:205-209 (1998). The reagent introduces breaks into the nucleic acid, thereby preventing its replication. The treated phage are subjected to purification methods to separate intact, replication competent phage from anti-bacterial phage that lack nucleic acid using *e.g.*, density separation..

h. Isolation of tails

[0148] Phage tails are most directly obtained from induction of a prophage-containing bacterial strain using mitomycin C or similar DNA-damaging chemicals. The prophage is induced and undergoes replication, while tails are assembled. No heads are synthesized because of a defect in a head assembly pathway. Thus, upon lysis, many tails are produced but no heads.

[0149] A similar "tails" production strategy involves isolating phage with conditional mutations in head gene expression or function. The mutant phage are grown up until the last step in production on suppressor hosts or under suppressing conditions. The simplest idea is to isolate temperature-sensitive mutants of the phage which have a temperature sensitive-mutation in an essential head gene. These mutant phages can be then grown under non-suppressing conditions, to generate the tail preparations.

i. Specific phage host combinations

[0150] Phage which have defects in genes necessary for packaging genetic material will produce phage assemblies which lack genetic material, *e.g.*, prophages. Variants can be isolated which exhibit mutations, *e.g.*, point, deletion, insertion, *etc.*, in critical head structure or head assembly genes, but, which do not affect production of the tail portions that are responsible for the binding and killing functions.

[0151] Lytic phage that are conditional producers of the nucleic acid packaging components can be isolated or engineered. For example, termination codons or temperature sensitive mutants can be identified to produce the phage particles under permissive conditions or in permissive hosts. Termination suppressor hosts or temperature sensitive hosts can allow production, while neither phage would be capable of replicating in the target bacterial hosts. Means to prevent transfer of the permissive factors into the phage genome are devised to minimize the possibility of recombination creating replication competent phage.

[0152] Alternatively, normal phage are produced in production hosts which are engineered to produce or assemble only the killing, *e.g.*, tail, components. This is achieved by blocking structural or assembly genes critical for assembly of the head components, *e.g.*, by plasmids that express antisense version of the target genes.

j. Combination of methods

[0153] The methods *a-i* can be combined to produce and enrich for replication incompetent phage, *i.e.*, anti-bacterial phage. In some embodiments, a separation step will be used to remove intact, replication competent phage. Further additional steps of nucleic acid removal or inactivation can be included to reduce the amounts of intact phage which may copurify with the replication incompetent anti-bacterial phage.

Example 2: Methods of determining anti-bacterial phage efficacy and dosage in animals.

[0154] Target bacteria, *e.g.*, *P. aeruginosa*, is grown in LB medium to an OD600 of 0.2, corresponding to 10^8 CFU/ml. After two rounds of 30 seconds of centrifugation at 12000 x g

and resuspension in phosphate-buffered saline (PBS), cells are diluted in PBS + 5% mucin to obtain 3x and 10x the minimal lethal dose (MLD) of bacteria per 100µl. Mice are inoculated intraperitoneally (IP) with 100µl of bacterial suspension. Controls and anti-bacterial phage dilutions in PBS are injected IP 45 min. after infection. Mice receive between 3 x 10^6 or 3 x 10^{10} killing units of anti-bacterial phage. Mice are allowed to eat and drink ad libitum throughout the 7 day observation period. Those of skill will recognize that dosages for humans can be extrapolated from the mouse dosages.

[0155] The following references also describe therapeutic administration of phage: Levin and Bull, *American Naturalist* 147:881-898 (1996); Barrow and Soothill, *Trends Microbiol.* 5:268-271 (1997); Eaton and Bayne-Jones, *JAMA* 103:1769-1776; 1847-1843; and 1934-1939 (1934); Smith and Huggins, *J. Gen. Microbiol.* 128:307-318 (1982); Smith and Huggins, *J. Gen. Microbiol.* 129:2659-2675 (1983).

WHAT IS CLAIMED IS:

1. A method of making an anti-bacterial phage, the method comprising the step of producing said anti-bacterial phage in a host bacterium, wherein said anti-bacterial phage is unable to replicate in a target bacterium and wherein said anti-bacterial phage inhibits growth of the target bacterium.

2. The method of claim 1, comprising a step of inactivating a nucleic acid of said anti-bacterial phage.

3. The method of claim 2, wherein the nucleic acid is inactivated by nicking the nucleic acid.

4. The method of claim 2, wherein the nucleic acid is inactivated by fragmenting the nucleic acid.

5. The method of claim 2, wherein the nucleic acid is inactivated by cross-linking said nucleic acid.

6. The method of claim 2, wherein the nucleic acid is inactivated by chemically modifying said nucleic acid.

7. The method of claim 1, comprising a step of removing a nucleic acid of said anti-bacterial phage.

8. The method of claim 7, wherein the nucleic acid is removed by osmotic shock.

9. The method of claim 7, wherein the nucleic acid is removed by a freeze thaw cycle.

10. The method of claim 7, wherein the nucleic acid is removed by chemical methods.

11. The method of claim 7, wherein the nucleic acid is removed by mechanical methods.

12. The method of claim 1, wherein a nucleic acid of said anti-bacterial phage comprises a mutation and cannot assemble into a replication competent phage, and

wherein the host bacterium is a complementing host bacterium that is able to complement the mutation of said anti-bacterial phage and allow replication of said anti-bacterial phage in the complementing host bacterium.

13. The method of claim 12, wherein the mutation is temperature sensitive at a non-permissive temperature, and wherein the complementing host bacterium complements the mutation at the non-permissive temperature.

14. A complementing host bacterium for use in the method of claim 12.

15. The method of claim 1, wherein a nucleic acid of said anti-bacterial phage comprises a mutation and cannot assemble into a replication competent phage, further comprising the step of supplying a complementing phage that can complement the mutation of said anti-bacterial phage and allow replication of said anti-bacterial phage in the host bacterium.

16. A pharmaceutical composition comprising an anti-bacterial phage, wherein said anti-bacterial phage inhibits growth of a target bacterium, and wherein said anti-bacterial phage has diminished replication activity in the target bacterium.

17. The composition of claim 16, wherein said anti-bacterial phage exhibits no replication activity in said target bacterium.

18. The composition of claim 16, wherein said anti-bacterial phage comprises less than 20% of the a nucleic acid content of an intact parental phage.

19. The composition of claim 18, wherein the anti-bacterial phage does not contain detectable nucleic acid.

20. The composition of claim 16, wherein said anti-bacterial phage comprises an intact phage comprising nucleic acid with reduced a replication capacity.

21. The composition of claim 16, wherein said anti-bacterial phage comprises a tail portion of a tailed phage.

22. The composition of claim 21, said anti-bacterial phage consists essentially of a tail portion of a tailed phage.

23. The composition of claim 16, further comprising a second anti-microbial agent.

24. The composition of claim 16, further comprising a therapeutically compatible buffer or excipient.

25. The composition of claim 16, wherein the anti-bacterial phage is made by a method comprising the steps of

a) amplifying a phage in a host bacterium,

b) harvesting the phage from the bacterial culture, and

c) removing substantially all of the nucleic acids from the phage, thereby producing the anti-bacterial phage.

26. The composition of claim 16, wherein the anti-bacterial phage is made by a method comprising the steps of

a) amplifying a phage in a host bacterium,

b) harvesting the phage from the bacterial culture, and

c) inactivating the nucleic acids of the phage, thereby producing the anti-bacterial phage.

27. The composition of claim 16, wherein the anti-bacterial phage is made by a method comprising the steps of

a) amplifying a phage in a host bacterium, and

b) harvesting the phage from the bacterial culture before an intact phage is produced, thereby producing the anti-bacterial phage.

28. The composition of claim 16, wherein the anti-bacterial phage is made by a method comprising the steps of

a) amplifying a phage in a host bacterium, and

b) harvesting the phage from the bacterial culture, wherein a nucleic acid of said anti-bacterial phage comprises a mutation and cannot assemble into a replication competent phage, and wherein the host bacterium is a complementing host bacterium that is able to complement the mutation of said anti-bacterial phage and allow replication of said anti-bacterial phage in the complementing host bacterium, thereby producing the anti-bacterial phage.

29. A method of treating a bacterial infection in a subject in need of such treatment, the method comprising administering a therapeutically effective amount of a compound of claim 16.

30. The method of claim 29, wherein the bacterial infection is caused by the target bacterium.

31. The method of claim 29, wherein the subject is a human.

32. The method of claim 29, wherein the subject is a primate, a food animal, or a companion animal.

33. The method of claim 29, wherein the bacterial infection is caused by *E. coli.*

34. The method of claim 29, further comprising administering a second antimicrobial agent.

35. The method of claim 29, wherein the compound is administered systemically.

36. The method of claim 35, wherein the compound is administered parenterally.

37. The method of claim 35, wherein the compound is administered orally.

38. The method of claim 29, wherein the compound is administered topically.

39. The method of claim 29, wherein the compound is administered by inhalation.

40. A pharmaceutical composition comprising a genetically incompetent anti-bacterial phage, wherein said anti-bacterial phage inhibits growth of a target bacterium.

41. The pharmaceutical composition of claim 40, wherein the genetically incompetent anti-bacterial phage lacks a full complement of genetic material.

42. The pharmaceutical composition of claim 40, wherein the genetically incompetent anti-bacterial phage has a mutation and cannot assemble into a replication competent phage in the target bacterium.

43. The pharmaceutical composition of claim 40, wherein the genetically incompetent anti-bacterial phage comprises nucleic acid with a reduced replication capacity.

44. The pharmaceutical composition of claim 40, wherein the genetically incompetent anti-bacterial phage consists essentially of a tail protein from a tailed phage.

45. A method of using a pharmaceutical composition of claim 40 to treat a bacterial infection in a subject in need of such treatment, the method comprising the steps of administering a therapeutically effective amount of the pharmaceutical composition.

46. The method of claim 45, wherein the subject is a human.

47. The method of claim 45, wherein the subject is a primate, a food animal, or a companion animal.

48. The method of claim 45, wherein the pharmaceutical composition is administered systemically.

49. The method of claim 45, wherein the pharmaceutical composition is administered topically.

50. The method of claim 45, wherein the pharmaceutical composition is administered in combination with an anti-bacterial agent.

51. A method of identifying an anti-bacterial phage that is unable to replicate in a target bacterium, the method comprising the steps of

a) identifying a target bacterium,

b) identifying a phage that can inhibit growth of the target bacterium, and

c) determining a form of the phage that is unable to replicate in the target bacterium.

52. An anti-bacterial phage that is identified using the method of claim 51, wherein the phage inhibits growth of a target bacterium and is unable to replicate in the target bacterium.

53. A method of producing an anti-bacterial phage comprising the steps of

a) amplifying a phage in a host bacterium,

b) harvesting the phage from the bacterial culture, and

c) removing substantially all of the nucleic acids from the phage, thereby producing the anti-bacterial phage.

54. The method of claim 53, wherein the nucleic acid is removed by osmotic shock.

55. The method of claim 53, wherein the nucleic acid is removed by a freeze thaw cycle.

56. The method of claim 53, wherein the nucleic acid is removed by chemical methods.

57. The method of claim 53, wherein the nucleic acid is removed by mechanical methods.

58. The method of claim 53, wherein the antibacterial phage is a tailed phage.

59. The method of claim 58, wherein the nucleic acids are removed by the steps of

a) separating a tail from a head of the tailed phage,

b) isolating the tail, thereby removing substantially all of the nuclei acids from the tailed phage.

60. The method of claim 58, wherein the nucleic acids are removed by the step of

a) harvesting the phage before a head and a tail have assembled to form an intact phage, and

b) isolating the tail, thereby removing substantially all of the nuclei acids from the tailed phage.

61. A method of making a defined dose anti-bacterial phage that kills a target bacterium, said method comprising at least one of the following steps:

a) producing said anti-bacterial phage in a host bacterium and isolating tail portions from DNA containing heads;

b) producing said anti-bacterial phage in a host bacterium and inactivating nucleic acid of said phage;

c) producing the anti-bacterial phage in a host bacterium and harvesting before complete assembly;

d) producing the anti-bacterial phage in a complementing host;

e) producing the anti-bacterial phage in a host bacterium with a helper phage; or

f) providing the anti-bacterial phage in a permissive host which phage are non-permissive in a different temperature.

62. The method of claim 61, wherein the anti-bacterial phage is a tailed phage.

63. The method of claim 62, wherein said isolating is after separation of phage tails from DNA containing phage heads.

64. The method of claim 61, wherein said inactivating is by at least one of the following methods:

a) nicking said nucleic acid;

b) fragmenting said nucleic acid;

c) crosslinking said nucleic acid;

d) chemically modifying said nucleic acid; or

e) removing said nucleic acid

f) harvesting said phage before complete assembly has occurred.

65. The method of claim 61, wherein the anti-bacterial phage is produced in a complementing host, wherein the anti-bacterial phage comprises at least one of the following mutations:

a) a point mutation; or

b) a deletion mutation; in a gene necessary for replication in said target bacterium.

66. The complementing host of claim 65, wherein said host encodes genes which complement the mutation in the anti-bacterial phage, thereby allowing the anti-bacterial phage to amplify in the host.

67. The method of claim 61, wherein the anti-bacterial phage exhibits less than 5% of the replication activity exhibited by intact phage in the host bacterium.

68. The method of claim 61, wherein the anti-bacterial phage exhibits diminished capacity to transmit toxin genes in the target bacteria when compared to intact phage in the host bacterium.

69. The method of claim 61, wherein the anti-bacterial phage exhibits diminished immunogenicity compared to intact phage from a host bacteria upon administration to a mammal.

70. The method of claim 61, wherein the anti-bacterial phage exhibits no detectable replication activity in the target bacterium.

71. The method of claim 61, wherein the target bacterium is a pathogenic bacterium.

72. The method of claim 71, wherein the pathogenic bacterium is a nosocomial or pyogenic bacterium.

73. The method of claim 71, wherein the pathogenic bacterium is a gram negative bacterium.

74. The method of claim 71, wherein the pathogenic bacterium is an *E. coli* bacterium.

75. A defined dose anti-bacterial composition comprising a phage protein derived from an intact parental phage, said antibacterial composition capable of killing a target bacterium, said antibacterial composition exhibiting less than 20% replication activity in the target bacterium, when compared to the intact parental phage.

76. The composition of claim 75, wherein said composition exhibits less than 5% replication activity in the target bacteriumwhen compared to the intact parental phage.

77. The composition of claim 75, wherein the anti-bacterial phage exhibits diminished capacity to transmit toxin genes in the target bacteria when compared to intact phage in the host bacterium.

78. The composition of claim 75, wherein the anti-bacterial phage exhibits diminished immunogenicity compared to intact phage from a host bacteria upon administration to a mammal.

79. The composition of claim 75, wherein the anti-bacterial phage exhibits no detectable replication activity in the target bacterium.

80. The composition of claim 75, herein the target bacterium is a pathogenic bacterium.

81. The composition of claim 80, wherein the pathogenic bacterium is a nosocomial or pyogenic bacterium.

82. The composition of claim 80, wherein the pathogenic bacterium is a gram negative bacterium.

83. The composition of claim 80, wherein the pathogenic bacterium is an *E. coli* bacterium.

84. The composition of claim 75, wherein said composition further comprises a nucleic acid with reduced replication capacity.

85 The composition of claim 84, wherein the nucleic acid has been nicked.

86. The composition of claim 84, wherein the nucleic acid has been fragmented.

87. The composition of claim 84, wherein the nucleic acid has been cross linked.

88. The composition of claim 84, wherein the nucleic acid has been UV irradiated.

89. The composition of claim 75, wherein said composition comprises less than 20% of the nucleic acid content of the intact parental phage.

90. The composition of claim 75, wherein said composition lacks detectable nucleic acid.

91. The composition of claim 75, wherein said composition comprises a damaged DNA is unable to be replicated.

92. The composition of claim 75, wherein the intact parental phage is a tailed phage and said composition comprises a tail.

93. The composition of claim 75, further comprising a second anti-microbial agent.

94. The composition of claim 93, wherein the second anti-microbial agent is an antibiotic.

95. The composition of claim 93, wherein the second anti-microbial agent is a bacterial cell wall growth disrupting compound.

96. The composition of claim 75, further comprising a therapeutically compatible buffer or excipient.

97. The composition of claim 75, wherein the anti-bacterial composition is made by a method comprising the step of processing the intact parental phage to remove or inactivate nucleic acids.

98. The composition of claim 75, wherein the anti-bacterial composition is made by a method comprising the step of harvesting phage from a host bacterium before intact phage are assembled.

99. The composition of claim 75, wherein the anti-bacterial composition is made by a method comprising the step of expressing in a complementing host strain a phage

genome defective in expressing critical genes for assembly, production, release, or infection by said phage.

100. The composition of claim 99, wherein the phage genome comprises one or more point mutations that prevents phage replication.

101. The composition of claim 99, wherein the phage genome comprises a deletion or insertion mutation that prevents phage replication.

102. A method of treating a bacterial infection comprising administering a composition of claim 75 to a eukaryote suffering from an infection by said target bacterium.

103. The method of claim 102, wherein the eukaryote is a mammal, including a primate.

104. The method of claim 102, wherein the target bacterium is a pathogenic, nosocomial, or pyogenic bacterium.

105. The method of claim 102, wherein the target bacterium is an *E. coli* bacterium.

106. The method of claim 102, wherein composition is administered topically.

107. The method of claim 102, wherein the infection has already been treated with an antibiotic.

108. The method of claim 102, wherein the infection has been diagnosed to be susceptible to the composition.

109. The method of claim 102, wherein the eukaryote is also inoculated with another bacterium to replace target bacterium.

110. A therapeutic anti-bacterial composition comprising a genetically incompetent phage wherein the genetically incompetent phage kills a target bacterium.

111. The composition of claim 110, wherein the genetically incompetent phage lacks detectable nucleic acid.

112. The composition of claim 110, wherein the genetically incompetent phage lacks a set of genes necessary to replicate in the target bacterium.

113. The composition of claim 110, wherein the genetically incompetent phage comprises a damaged nucleic acid.

114. The composition of claim 110, wherein the genetically incompetent phage comprises a tail protein from a tailed phage.

115. The composition of claim 110, wherein the composition is used therapeutically to treat a companion animal, food animal, or primate.

116. The composition of claim 110, wherein the target bacterium is a pathogenic bacterium.

117. A method of using a composition of claim 110, wherein the composition is administered topically.

118. A method of using a composition of claim 110, wherein the composition is administered systemically.

119. A method of using a composition of claim 110, wherein the composition is administered in combination with a second anti-bacterial agent.

120 The method of claim 119, wherein the second anti-bacterial agent is an antibiotic.

DEFINED DOSE THERAPEUTIC PHAGE

ABSTRACT OF THE DISCLOSURE

The invention provides therapeutic, defined-dose anti-bacterial phage preparations, methods to make such preparations, methods to treat bacterial infections using such preparations and method to diagnose bacterial infections using such preparations.

Application Data Sheet

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